

Application No.: 10582820
Amdt. Dated May 28, 2010
Second Reply to Office Action of March 29, 2010

Amendments to the Specification:

Please amend the Specification as follows:

Please replace paragraph [0011] with the following amended paragraph:

[0011]FIGS. 1 (a)-(d) A-D depict assembly of the molecular semaphore of the invention.

Please replace paragraph [0012] with the following amended paragraph:

[0012]FIGS. 2 (a)-(b) A-B depict rotation of the molecular semaphore of the invention.

Please replace previously amended paragraph [0016] with the following amended paragraph:

[0016] FIG. [[6a]] 6A and FIG. [[6b]] 6B provide a summary of experimental results regarding DNA sequence specificity.

Please replace paragraph [0059] with the following amended paragraph:

[0059]As shown in FIG. [[1]] 1A-FIG. 1D, one embodiment of the disclosed methods comprises the following steps:

Please replace paragraph [0060] with the following amended paragraph:

[0060]1. As indicated in FIG. [[1a]] 1A a, a first affinity tag is attached to the 5' end of the first target-specific DNA strand. A second affinity tag is attached to the 3' end of second target-specific DNA strand (FIG. [[1a]] 1A).

Please replace paragraph [0061] with the following amended paragraph:

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[0061]2. In FIG. [[1b]] 1B the first and second target-specific nucleic acid strands are hybridized to the target nucleic acid so that the 3' end of the first target-specific strand is directly adjacent to the 5' end of the second target-specific strand.

Please replace paragraph [0062] with the following amended paragraph:

[0062]3. In FIG. [[1c]] 1C the first and second target-specific DNA strands are ligated, to generate a double-stranded DNA sequence that contains the first and second affinity tags at each end.

Please replace paragraph [0063] with the following amended paragraph:

[0063]4. In FIG. [[1d]] 1D the double-stranded DNA that contains the affinity tags is then used as a bridge between a molecular motor and the detection probe used to detect the motion generated from the motor via the affinity tags. The first affinity tag attaches specifically to a moiety on a moving component of the molecular motor while the second affinity tag is specific to the detection probe. In the specific preferred and exemplary embodiment the first affinity tag is an avidin link between a moiety on the motor receptor and the first target-specific nucleic acid. The motor shown is the F₁-ATPase biomolecular motor. In the specific example, the detection probe comprises gold nanorods that can be visualized by microscopy, attached to the second target-specific nucleic acid by a biotin bond formed. The particular gold rods were of a size and were illuminated such that regularly changing color change from red to green and back, characteristically indicated rotation of the rods.

Please replace paragraph [0064] with the following amended paragraph:

[0064]5. Immobilization occurs after assembly of the components in step 4 (FIG. [[2a]] 2A). Immobilization is effected by histidine binding of the nonrotational F1 motor structure to a nickel surface. 6. The movement of the molecular motor is induced by

adding ATP (FIG. [[2b]] 2B). The only motion that will be detected will result from motors that are connected to the attached detection probe. Since that will depend upon the presence of the double-stranded DNA bridge and because the double stranded DNA can only result from the specific hybridization of the three single strands, observation of this motion will identify the presence of the target strand of DNA.

Please replace previously amended paragraph [0141] with the following amended paragraph:

[0141] FIG. [[6a]] 6A and FIG. [[6b]] 6B provide a summary of experimental results regarding DNA sequence specificity. For any detection method, it is necessary to minimize the number of false positives. DNA detection relies upon the inherent complementary base pairing properties of the molecule. To optimize detection sensitivity, therefore, it is important to minimize the number of DNA bridges formed in the presence of base-pair mismatches between the probes and target molecule. LCR is sensitive to mismatches several bases away from the ligation site. Mismatches prevent ligation from occurring to form the biotinylated DNA bridges. This is represented on the sequence specificity graph, where the number of bridges formed with a mismatched target is dramatically lower than a fully complementary target.